

# 5,6-dihydroxyindole-2-carboxylic acid (DHICA): a First Principles Density-Functional Study

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We report first principles density functional calculations for 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and several reduced forms. DHICA and 5,6-dihydroxyindole (DHI) are believed to be the basic building blocks of the eumelanins. Our results show that carboxylation has a significant effect on the physical properties of the molecules. In particular, the relative stabilities and the HOMO-LUMO gaps (calculated with the  $\Delta$ SCF method) of the various redox forms are strongly affected. We predict that, in contrast to DHI, the density of unpaired electrons, and hence the ESR signal, in DHICA is negligibly small.

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## I. INTRODUCTION

The melanins are an important class of pigmentary macromolecule found throughout nature from fungi to man [1]. Two types of melanin are found in the skin, hair, eyes and nervous systems of humans: pheomelanin and eumelanin. Pheomelanin is associated with ginger hair and is a cysteinyl-dopa derivative. Eumelanin, which we consider here, is found in much higher levels in black hair and is believed to be a macromolecule of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and 5,6-dihydroxyindole (DHI) [1, 2]. Perhaps the most surprising property of melanins is that, whereas most biomolecules show only well defined absorption peaks around 280 nm (4.5 eV) and little absorption at lower energies [4], the melanins show broad band monotonic absorption in the range 1.5 to 5 eV [3]. Melanins are also efficient free radical scavengers and antioxidants [1]. Thus melanins are ideally suited for their best understood biological function as the primary photoprotectant in our skin and eyes. Conversely, both pheomelanin and eumelanin are implicated in the development of melanoma skin cancer [5]. Melanins also display semiconductor like behaviour [6, 7, 8] that suggests possible uses in applications such as bio-sensors and bio-mimetic photovoltaics [9, 10]. However, it is not clear that the conductivity reported in any of these experimental studies is electronic in nature [10, 11].

Surprisingly, given the obvious importance of the melanins and several decades of work, little is known about the general structure-property-function relationships that control their behaviour. This is, at least in part, due to the difficulties in studying the melanins; they are chemically and photochemically stable, and are virtually insoluble in most common solvents. For example, major questions still remain concerning their basic structural unit [12]. Two opposing schools of thought exist: i) that eumelanins are composed of highly cross-linked

extended hetero-polymers based upon the Raper-Mason scheme [1], and ii) that eumelanins are actually composed of much smaller oligomers condensed into 4 or 5 oligomer nano-aggregates [13]. A clear idea of the basic structural unit is critical to developing a consistent model for condensed phase charge transport in such disordered organic systems. It is also important in the context of “molecularly engineering” melanins to have the ability to create or enhance functionality in high technology applications [9, 10].

Recently the role of disorder in determining the physical properties of the melanins has been emphasised [10, 14, 15]. In particular it has been proposed that the broad band monotonic optical absorption of eumelanin arises from the existence of a large number of different monomers and hence a wide variety of macromolecules. The idea is, basically, that, although each of these molecules has a distinct optical absorption that is not atypical for bio-macromolecule, summing over a large range of absorption frequencies in a biological environment (in which both dipole fluctuations [16] and thermal effects broaden the spectrum) gives rise to the observed broad band monotonic absorption spectrum.

Despite the widespread agreement that natural eumelanin contains both DHI and DHICA, previous quantum chemical studies [14, 15, 17, 18, 19, 20, 21, 22, 23] have only considered DHI. This is in part due to the greater chemical complexity of DHICA, but also in part due to assumption that the carboxylation of DHI does not play a significantly role in determining the properties eumelanin. However, given the potential importance of the role of disorder in the physical properties of eumelanin [10, 14, 15] a careful study of the similarities and differences between DHI and DHICA is clearly required. Our study is also motivated by recent work on synthetic DHICA [24] and the need for high quality first principles calculations to compare with future experiments on synthetic DHICA. In this paper we present, to our knowledge, the first quantum chemical study of DHICA. We use density functional theory (DFT) to calculate the physical and electronic structure of DHICA and several reduced forms, each of which is drawn schematically in

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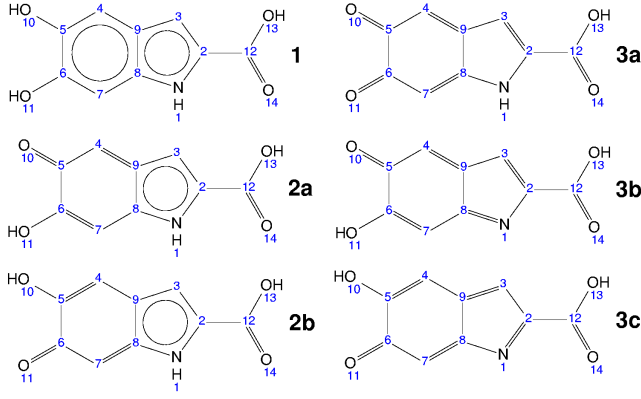


FIG. 1: Schematic representation of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) **1** and the reduced forms which we also consider here. The numbers correspond to those used in table I. Note that **2a** and **2b** are radicals in the neutral state.

figure 1. Our results show that there are significant differences between the physical properties of DHI and DHICA. Specifically we show that the relative stability of the reduced forms is significantly altered by carboxylation as is the HOMO-LUMO gap [the energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO)].

## II. CALCULATION DETAILS

The chemical and electronic structures were found from first principles DFT calculations. We have performed our calculations using the Naval Research Laboratory Molecular Orbital Library (NRLMOL) [25, 26, 27, 28, 29, 30, 31]. NRLMOL performs massively parallel electronic structure calculation using gaussian orbital methods. Throughout we have used the Perdew, Burke and Ernzerhof (PBE) [32] exchange correlation functional, which is a generalised gradient approximation (GGA) containing no parameters. For each molecule we have fully relaxed the geometry with no symmetry constraints.

## III. RESULTS AND DISCUSSION

We report the calculated bond lengths and bond angles of DHICA in table I. For comparison we also report the same quantities for DHI from calculations [15] using the same methods (it should be noted that the results for DHI are in good agreement with other quantum chemical calculations [21, 22, 23]). Unsurprisingly there is little difference between the physical structure of DHI and DHICA.

In table II we report the calculated energies of each of the molecules considered here in their neutral, -1 and -2 charge states. We also report the relative concentration at 300 K in table III. It can be seen that in the neutral

TABLE I: The calculated bond lengths (in Å) and bond angles (in degrees) of DHICA. The atom numbers correspond to those shown in figure 1. Note that there is no experimental data or previous theoretical results to compare with. We therefore contrast our results with previously published results for DHI [15].

	DHICA	DHI
N1-C2	1.387	1.390
C2-C3	1.388	1.365
C3-C9	1.421	1.443
C9-C4	1.414	1.366
C4-C5	1.380	1.458
C5-C6	1.428	1.586
C5-O10	1.384	1.226
C6-O11	1.365	1.226
C6-C7	1.387	1.457
C7-C8	1.403	1.361
C8-C9	1.432	1.483
C8-N1	1.372	1.381
C2-C12	1.453	-
C12-O13	1.368	-
C12-O14	1.225	-
N1-C2-C3	109.1	110.8
C2-C3-C9	107.0	107.2
C3-C9-C4	133.5	132.7
C3-C9-C8	107.2	106.2
C9-C4-C5	118.3	119.7
C4-C5-C6	121.7	118.3
C4-C5-O10	124.8	122.7
O10-C5-C6	113.6	118.8
C5-C6-O11	118.9	119.1
C5-C6-C7	121.2	117.9
O11-C6-C7	119.8	122.8
C6-C7-C8	117.2	118.3
C7-C8-C9	122.3	124.6
C8-C9-C4	119.3	121.0
N1-C8-C9	107.3	106.0
C7-C8-N1	130.5	129.3
C8-N1-C2	109.4	109.6
N1-C2-C12	118.9	-
C3-C2-C12	132.0	-
C2-C12-O13	112.9	-
C2-C12-O14	124.2	-
O13-C12-O14	112.8	-

state DHICA is considerably more stable than any of the reduced forms. Whereas in the -1 state **2a** and **2b** are most stable. This result has important implications for electron spin resonance (ESR) experiments. ESR experiments are sensitive to unpaired electrons which, these calculations indicate, are not found in DHICA (this is not the case for DHI [15, 23]). Given the recent synthesis of DHICA, ESR experiments on pure DHICA samples would provide a direct check of these calculations. These results suggest that samples of DHICA have significantly less chemical disorder than pure DHI or natural eumelanin. We can therefore speculate that the optical absorption of macromolecules formed from synthetic DHICA may not be as smooth as that of natural eume-

TABLE II: The total energy (in eV) of DHICA and the reduced forms shown in figure 1 in the neutral, -1 and -2 charge states. The energies are quoted relative to the energy of neutral DHICA. In each case we fully relaxed the geometries as shown in figure 2.

	Neutral	-1	-2
<b>1</b>	0	-0.299	+3.245
<b>2a</b>	+1.165	-1.478	+1.887
<b>2b</b>	+1.152	-1.613	+1.813
<b>3a</b>	+2.392	-0.223	+1.814
<b>3b</b>	+2.693	-0.307	+1.273
<b>3c</b>	+2.583	-0.321	+1.276

TABLE III: The relative abundances of DHICA and the reduced forms shown in figure 1 in the neutral, -1 and -2 charge states at 300 K. We have assumed a Boltzmann distribution of each charge state separately with an infinite reservoir of  $H_2$ . The relevant energies are stated in table II.

	Neutral	-1	-2
<b>1</b>	1	$< 10^{-22}$	$< 10^{-33}$
<b>2a</b>	$< 10^{-19}$	$5.5 \times 10^{-3}$	$< 10^{-10}$
<b>2b</b>	$< 10^{-19}$	1	$< 10^{-9}$
<b>3a</b>	$< 10^{-40}$	$< 10^{-23}$	$< 10^{-5}$
<b>3b</b>	$< 10^{-45}$	$< 10^{-21}$	1
<b>3c</b>	$< 10^{-44}$	$< 10^{-21}$	0.88

lanin. Note that for all of the molecules considered here the -1 charge state is more stable than the neutral state (as is the case for the related DHI molecules [14, 15]), this is clearly related to free radical scavenging properties of eumelanin.

One of the most important properties of melanin

TABLE IV: The HOMO-LUMO (highest occupied molecular orbital-lowest unoccupied molecular orbital) gap in eV. The HOMO-LUMO gap has been calculated from both the  $\Delta$ SCF method and from a simple interpretation of the Kohn-Sham eigenvalues. We have previously shown [10, 15] that for DHI and related molecules the value of the HOMO-LUMO gap calculated by  $\Delta$ SCF method is in good agreement with the results of time dependent density functional theory (TDDFT) calculations for the same molecules [23]. The figures in brackets show the results of equivalent calculations for DHI [15]. It can be seen that there is a significant change in the HOMO-LUMO gap for **1**, which is essentially the only stable monomer in the neutral state (c.f. tables II and III).

	Simple interpretation of the Kohn-Sham eigenvalues	$\Delta$ SCF
<b>1</b>	2.85 (3.48)	3.04 (3.61)
<b>2a</b>	2.24 (-)	2.67 (-)
<b>2b</b>	2.37 (-)	2.64 (-)
<b>3a</b>	0.87 (1.07)	1.96 (2.02)
<b>3b</b>	0.79 (0.80)	1.10 (1.12)
<b>3c</b>	0.89 (-)	1.25 (-)

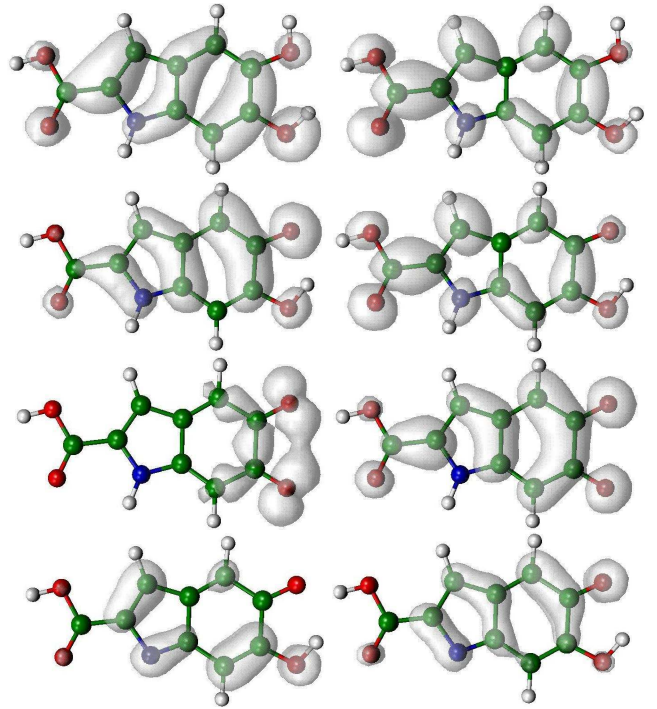


FIG. 2: (Colour online.) The electron density in the highest occupied molecular orbital (HOMO) (left) and the lowest unoccupied molecular orbital LUMO (right) of, from the top down, **1**, **2a**, **3a** and **3b**. Although the electron density in the HOMO of **1** is very similar to the electron density in the HOMO of DHI (reported in [15]) there are significant differences between the electron densities in the LUMOs of DHICA and DHI near atom C2 (where the carboxylic group attaches). This is presumably related to the large difference ( $\sim 20\%$ ) in HOMO-LUMO gap calculated for the two compounds (c.f. table IV). Both the HOMO and LUMO electron densities of **3a** and **3b** are very similar to the electron density in the HOMO and LUMO of the equivalent reduced forms of DHI (the indolequinone and the semiquinone respectively, see [15]) This is presumably related to the similarity of HOMO-LUMO gap calculated for the carboxylated and uncarboxylated forms of the two compounds (c.f. table IV).

monomers is the HOMO-LUMO gap. This is indicative of the optical spectrum as it represents the fundamental optical absorption and Hückel model calculations [18] for DHI show that the HOMO-LUMO gap of a single molecule correlates with the semiconducting gap of an infinite homopolymer. The “semiconductor” gap is likely to be a critical design parameter for molecularly engineered forms of eumelanin which may be useful as functional materials in electronic devices and sensors [9, 10]. Thus controlling the HOMO-LUMO gap of monomers and thence of macromolecules may provide a route to controlling the semiconducting gap of eumelanins [15].

DFT is a theory of the ground state, therefore calculations represent the energy gap between Kohn-Sham eigenvalues and not the true HOMO-LUMO gap of the molecules. This is known as the band gap problem [33]. Additionally, it is accepted that the PBE functional

can significantly underestimate the HOMO-LUMO gap. Therefore we have also employed the  $\Delta$ SCF method [33] to calculate the HOMO-LUMO gap. We have previously shown that equivalent results for DHI reproduce the trends found in time dependent DFT calculations [23]. In table IV we compare the HOMO-LUMO gap found from a simple interpretation of the Kohn–Sham eigenvalues with those found by the  $\Delta$ SCF method. We also reproduce the equivalent results for some of the uncarboxylated forms from [15]. From this it can be seen that for **3a** and **3b** carboxylation does not significantly change the HOMO-LUMO gap. But, for **1**, which our results show is the only significant component of DHICA samples (table III), carboxylation causes a significant change in the HOMO-LUMO gap. This indicates that, because natural eumelanin contains both DHI and DHICA, a sample of eumelanin contains a large range of HOMO-LUMO gaps, even before the effects of the formation of macromolecules are considered.

The changes in the HOMO-LUMO gap in DHI are understood in terms of the delocalisation of the wavefunction. Therefore in figure 2 we plot the calculated electron density of **1**, **2a**, **3a** and **3b** respectively. Comparing these plots with the equivalent results for the uncarboxylated forms [15] we see that for **3a** and **3b** the electron densities in both the HOMOs and LUMOs is remarkably similar in both the carboxylated (figure 2) and uncarboxylated forms. This is presumably the reason that the calculated HOMO-LUMO gap is essentially independent of carboxylation (table IV). On the other hand while the electron density of the HOMO of **1** is very similar to that of its uncarboxylated form, there are significant differences between the electron density of the LUMO and that of the uncarboxylated form (figure 2). This is, presumably, associated with the large change in the HOMO-LUMO gap caused by carboxylation (c.f. table IV).

#### IV. CONCLUSIONS

We have carried out first principles density functional calculations for 5,6-dihydroxyindole-2-carboxylic

acid (DHICA) and several reduced forms. To the best of our knowledge these are the first quantum chemical calculations for this system. These molecules and similar molecules based on 5,6-dihydroxyindole (DHI) are believed to be the basic building blocks of the eumelanins. We used the  $\Delta$ SCF method to study the HOMO-LUMO gap. Comparing our results for DHICA with previously published results for DHI we found that the addition of the carboxylic acid group has a significant effect on the physical properties of the molecules and in particular on the relative stability of the various redox forms and the HOMO-LUMO gap. Based on the calculated relative stabilities of the various redox forms in their various charge states we find predict that there will be an extremely low unpaired electron density in samples of pure DHICA (this is not the case for DHI [14, 15]) and thus a negligible signal should be seen in ESR experiments. This indicates that experimental results cannot be straightforwardly extrapolated from DHICA to DHI or *vice versa*.

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